IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of **Goddard et al.**

Serial No. 09/944,929

Filed: August 31, 2001

Title: **SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME**

Examiner: Nancy Vogel

Group Art Unit: 1636

Confirmation No. 1971

Attorney's Docket No. 10466/140

DECLARATION OF AUDREY GODDARD, Ph.D., PAUL J. GODOWSKI, Ph.D., J. CHRISTOPHER GRIMALDI, AUSTIN L. GURNEY, Ph.D., DANIEL TUMAS, Ph.D. AND WILLIAM I. WOOD, Ph.D. UNDER 37 CFR § 1.131

MAIL STOP AMENDMENT
The Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, Audrey Goddard, Ph.D., Paul J. Godowski, Ph.D., J. Christopher Grimaldi, Austin Gurney, Ph.D., Daniel Tumas, Ph.D. and William I. Wood, Ph.D. declare and say as follows:

1. We are the inventors of the above-identified application.

2. At the time the present invention was made, one of the inventors, Daniel Tumas, Ph.D., was responsible for overseeing the testing of novel polypeptides, including the polypeptide PRO361, in an assay of inhibitory activity in the mixed lymphocyte relation (MLR) (Assay #67, Example 34). This assay is used to find agents that are active as inhibitors of the proliferation of stimulated T-lymphocytes. Compounds which inhibit proliferation of lymphocytes are useful therapeutically where suppression of an immune response is beneficial.

3. The basic protocol for this assay is described in Current Protocols in Immunology, unit 3.12, edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO_2) and then washed and resuspended to $3x10^6$ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a misture of:

100:1 of test sample diluted to 1% or to 0.1%,

50:1 of irradiated stimulator cells, and

50:1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO_2 for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mC/well; Amersham). After 6 hours the cells are washed 3 times and then the update of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to $1x10^7$ cells/ml of assay media. The assay is then conducted as described above.

Any decrease below control is considered to be a positive result for an inhibitory compound, with decreases of less than or equal to 80% being preferred. However, any value less than control indicates an inhibitory effect for the test protein. The results are

value less than control indicates an inhibitory effect for the test protein. The results are indicative of the utility of the PRO polypeptides in therapeutic applications where suppression of an immune response is beneficial.

4. Copies of pages from an internal database showing the positive results for the PRO361 polypeptide (SEQ ID NO: 83), identified by Pin number PIN996-1, in Assay #67 are attached to this declaration (with dates redacted) as Exhibit A. These experiments were performed and the results were obtained in the United States prior August, 1999.

5. Exhibit A clearly shows that the polypeptide designated PRO361 was tested, and its ability to inhibit the mixed leukocyte reaction was determined prior to August, 1999.

6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

A. Goddard — 1/12/08

Audrey Goddard, Ph.D. — Date

Paul J. Godowski, Ph.D. — Date

J. Christopher Grimaldi — Date

Austin L. Gurney, Ph.D. — Date

Daniel Tumas, Ph.D. — Date

William I. Wood, Ph.D. — Date

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Audrey Goddard, Ph.D.	Date
Paul J. Godowski, Ph.D.	12/19/07 Date
J. Christopher Grimaldi	Date
Austin L. Gurney, Ph.D.	Date
Daniel Tumas, Ph.D.	Date
William I. Wood, Ph.D.	Date

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Audrey Goddard, Ph.D.	Date
Paul J. Godowski, Ph.D.	Date
J. Christopher Grimaldi	Date
Austin L. Gurney, Ph.D.	1/8/13 Date
Daniel Tumas, Ph.D.	Date
William I. Wood, Ph.D.	Date

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Audrey Goddard, Ph.D.	Date
Paul J. Godowski, Ph.D.	Date
J. Christopher Grimaldi	Date
Austin L. Gurney, Ph.D.	Date
Daniel Tumas, Ph.D.	February 14, 2008 Date
William I. Wood, Ph.D.	Date

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Austin L. Gurney, Ph.D.	Date
Daniel Tumas, Ph.D.	Date
William I. Wood, Ph.D.	12/14/07 Date

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Audrey Goddard, Ph.D.	Date
Paul J. Godowski, Ph.D.	Date
J. Christopher Grimaldi	3/31/06 Date
Austin L. Gurney, Ph.D.	Date
Daniel Tumas, Ph.D.	Date
William I. Wood, Ph.D.	Date

EXHIBIT A

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SEARCH | REQUEST SYSTEMS | ANALYSIS TOOLS | RESOURCES | Historical SPDI Record

Quick search: DNA | Enter id number | Spdi | Gene | Seq

Assay Viewer

SPDI Assays

ASY57 Rod Photoreceptor Cell cAMP Synergy(2 Da
ASY58 Rat DRG Neuronal Survival Inhibition Ass
ASY59 Ventralization of Xenopus Embryo by DNA
ASY60 Benzidine Staining of Xenopus Embryo by
ASY61 Ventralization of Xenopus Embryo by RNA
ASY62 Benzidine Staining of Xenopus Embryo by
ASY63 Hematopoiesis: stem cell proliferation
ASY64 Proinflammatory/PMN Infiltrate
ASY65 HUVEC Survival in Absence of Serum
ASY66 Inhibition of Adipocyte Differentiation
ASY67 MLR - Inhibitory

Select All | Unselect

All Positives | Verified Positives | Pending

Date Complete From To

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LOT

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2176

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Search

Lots for Search

PIN996-1

Remove | Remove All

PurSearch | DNA Search | Reset

ASSAY RESULT LIST Rows 1 - 4 of 4 Page 1 of 1 Select Page Page No. 1 Go

ASY	ASY Name	PUR/EXP/DNA	LOT	LOT Name	Pos	Verified	Conc	Conc Unit	Mean	Crd	UNQ	Protein Name	Date Distr	Date Complete	Comment
ASY67	MLR-Inh	PUR1292	LOT2176	PIN996-1			1.50	nM	63.2		UNQ316	Human MANSC1 IgG			
ASY67	MLR-Inh	PUR1292	LOT2176	PIN996-1			1.50	nM	77.3		UNQ316	Human MANSC1 IgG			
ASY67	MLR-Inh	PUR1292	LOT2176	PIN996-1			15.00	nM	64.7		UNQ316	Human MANSC1 IgG			
ASY67	MLR-Inh	PUR1292	LOT2176	PIN996-1			15.00	nM	99.4		UNQ316	Human MANSC1 IgG			

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